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ORIGINAL ARTICLE

Malaria: Patterns of relapse and resistance

M.A. Saifi [a], Wajihullah [b], M.I. Siddiqui [a], M.S. Al-Khalifa [a,*]

[a] *Department of Zoology, College of Science, P.O. Box 2455, King Saud University, Riyadh-11451, Saudi Arabia*
[b] *Department of Zoology, Aligarh Muslim University, Aligarh 202 002, India*

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Abstract Malaria constitutes persistent threat to the human health and remains a distinct cause of morbidity and mortality, 40% of the world population is at risk of exposure to this menace in 100 countries (WHO, 2001). Present data represents the registered cases of malaria in the hospitals and clinics in India. *Plasmodium vivax* and *Plasmodium falciparum* infections were recorded 63.86%, 66.06%, 62.58% and 36.13%, 33.93%, 37.41%, respectively, amongst individuals with symptoms of intermittent high fever for three days. Maximum transmission with highest slide positivity rates (SPR) 61.76% and 50.14% was periodically and strategically observed during September and October while lowest yearly transmission slide positivity rates (SPR) (19.60–24.07%) was estimated in the months of March and April. Average relapse rates (ARR) in *P. vivax* was recorded 17.1%. Short term relapses were more recurring than the long term in the ratio of 4:1. Eighty-eight patients who were administered with total of 1500 mg chloroquine and 75 mg primaquine through divided doses also showed relapse rate of 4.5%. Patients suffering from *falciparum* malaria showed resistance against chloroquine in 10.6% cases after getting 1500 mg chloroquine based on divided doses (i.e. 600 mg on day 1 and 300 mg after 8 h and followed by 300 mg daily for 2 days.)

1. Introduction

Malaria is a major burden for most resource-poor nations of the world. Between 200 and 500 million deaths attributable to malaria, most among the children of sub-Saharan Africa (Breman, 2001). In fact, 9 out of 10 cases of malaria occur in this region, while two third of remaining are concentrated in just six countries viz., India, Brazil, Sri Lanka, Vietnam, Cambodia and Solomon Island. The goal of eradicating malaria, once thought to be possible, was abandoned decades ago, and the present goal of malaria control is first to retard the accelerating rates of disease and death and then to "Roll Back Malaria".

In 1990s malaria re-emerged and took the lives of several thousand people (Sharma, 1996). Factors responsible for the re-emergence of malaria were vector resistance to insecticide and parasite's resistance to drug. In India 60–65% of the malaria infections are reported to be due to *Plasmodium vivax* and 30–35% due to *Plasmodium falciparum* (Adak et al. 1998). The worsening problems of drug resistance in many parts of the world and the limited number of antimalarial drugs available has increased difficulties for the development of anti-malarial drug policies and the provision of adequate disease management. It is now recognized that most endemic countries will

* Corresponding author.
E-mail address: mkhalifa@ksu.edu.sa (M.S. Al-Khalifa).

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have to face the unavoidability of some resistance to the antimalarial drugs used to treat uncomplicated malaria.

In India resistance of *P. falciparum* to chloroquine, the cheapest and the most frequently used therapeutic drug was first reported in the year 1973 from Diphu of Karbi-Analog district in Assam state. The reports on the resistance of *P. falciparum* to chloroquine and amodiaquine, first observed in 1960–1961 in Colombia and in Brazil (Bruce-Chwatt, 1985), were of greater consequence, since together with quinine these are the most valuable drugs for the treatment of acute malaria. Further reports on drug resistance came from Thailand, Malaysia, Cambodia, Phillipines, Indonesia, Vietnam, Laos, Burma and other areas of South-East Asia. Available information indicates that *P. falciparum* has given rise to formidable drug resistant strain in Asia. The problem of chloroquine resistance in *P. falciparum* is widespread and now it is leading towards multiple drug resistance against all major antimalarials (WHO, 1996).

2. Materials and methods

Present study is based on the malaria cases enrolled in Jawaharlal Nehru Medical College and a few other hospitals and clinics of Aligarh during the years 2001, 2002 and 2003. Blood smear were prepared from patients who attended hospitals and clinics, complained for fever and headache and were suspected of malaria. Thin and thick blood smears of patients were prepared by finger prick, stained with J.S.B. and Giemsa stains and microscopically examined under an oil immersion lens to see the positivity for malaria infection. From malaria positive cases, monthly prevalence of *P. vivax* and *P. falciparum* infections were recorded. Month-wise slide positivity rates (SPR) and slide *falciparum* rates (SFR) were worked out for the years 2001, 2002 and 2003. Resistant and relapse cases were also observed in *P. falciparum* and *P. vivax* infections, respectively.

For the study of relapse in *P. vivax,* one group of patients was treated by giving 1500 mg chloroquine base in divided doses (i.e. 600 mg on day 1 and 300 mg after 8 h followed by 300 mg daily for 2 days). The dose of child was adjusted according to his/her body weight. While patients in other group were administered 1500 mg chloroquine in the similar manner followed by 15 mg primaquine daily for 5 days and then followed up carefully. To determine the pattern of relapse in *P. vivax* each patient was identified individually by name, address and subsequent treatment. On reporting back blood smears were prepared from the patient and examined microscopically for the presence of malaria parasite and entered against his/her name.

The following criteria were used in classifying the patients into primary cases and non-relapse and relapse categories in the present study. Patient reporting for the first time (having no history of malaria) with acute illness and showing symptoms such as high fever, severe headache, loss of appetite, occasional vomiting and microscopic evidence of *P. vivax* infection were considered as primary cases. Some patients in this group who had no clinical symptoms of malaria or parasitological evidence of *P. vivax* infection following their primary infection during the entire period was considered as non-relapse cases. Those patients who reported back to the clinic within one month to one year with renewed clinical symptom (mild) along with a periodic alternate day fever (not observed in the primary cases) and found to be microscopically positive for *P. vivax* infection were considered as relapse cases. After medication if patient again suffered from malaria within 3 months with more regular paroxysm, he/she was treated as a case of short term relapse. But if it happened beyond 3 months then the case was considered as long term relapse. Cases of *P. vivax* who did not respond to 1500 mg of chloroquine and 75 mg primaquine was recorded as chloroquine resistant cases.

2.1. Drug resistance in *P. falciparum* infections

For drug resistance, patients who were positive for *P. falciparum* infections were given 1500 mg chloroquine base in divided doses (i.e. 600 mg on day 0, 300 mg after 8 h followed by 300 mg daily for 2 days). The dose of child was adjusted according to his/her body weight. Blood films of those patients who reported back with fever within 4 weeks of treatment were examined for *P. falciparum* infection. If found positive, such cases were recorded as chloroquine resistant cases. Level of resistance (i.e. RI, RII, RIII) was ascertained on the basis of late and early recrudescence.

3. Results

Tables 1–3 show month wise slide positivity rates and slide *falciparum* rates for the years 2001–2003 for *P.* vivax and *P.* falciparum infections observed in Aligarh. These tables also provide information on seasonal fluctuations of aforesaid species. *P.* vivax infection was predominant and was recorded in all months of the year, with almost similar seasonal patterns during the three successive years. *P.* vivax showed a gradual increasing trend from July onwards reaching a peak in September soon after the rainy season, and then showed a decline in December which continued until June. *P.* falciparum infections started gradual increasing in July–August and showed a peak in September and October and then decline November onwards with the on set of winter and continued to decline till June.

During 2001 out of 1473 slides examined, 487 were found positive for malaria of which 311 belonged to *P. vivax* and 176 to *P. falciparum*. In 2002 a total of 1369 slides were examined, out of which 498 were found positive for malaria of which 329 belonged to *P. vivax* and 169 to *P. falciparum*. In 2003 a total of 1428 slides were examined for malaria infection, out of which 580 were found positive. Slides showing positivity for *P. vivax* and *P. falciparum* were 363 and 217, respectively. During 2001–2003 overall percentage of *P. vivax* and *P. falciparum* were 63.86%, 66.06%, 62.58% and 36.13%, 33.93% and 37.41%, respectively (Table 4).

It was observed that malaria transmission was least from January to April. During the months of May, June and July transmission was low with slight fluctuating figures for the years 2001–2003 which was in accordance with the commencement of pre-monsoon shower that contributed slightly increased or decreased transmission rate in proceeding months. Increased rate of transmission was recorded July onwards, reaching a peak soon after rains in September and October followed by a sharp decline to a low level in December. During peak transmission season mean temperature and relative humidity ranged somewhere around 26–28 °C and 77–88%.

Table 1 Prevalence of malaria showing parasite distribution, SPR and SFR during year 2001.

Month	BSE	Total cases	*P. vivax*	*P. falciparum*	SPR	SFR
Jan	27	8	6	2	29.62	7.40
Feb	32	8	5	3	25.00	9.35
March	47	11	7	4	23.40	8.51
Apr	54	13	8	5	24.07	9.25
May	62	12	8	4	19.35	6.45
June	55	11	7	4	20.00	7.27
July	74	27	21	6	36.48	8.10
Aug	135	28	19	9	20.74	6.66
Sept	382	170	108	62	44.50	16.23
Oct	351	129	78	51	36.75	14.52
Nov	157	50	32	18	31.84	11.46
Dec	97	20	12	8	20.61	8.24

Table 2 Prevalence of malaria showing parasite distribution, SPR and SFR during year 2002.

Month	BSE	Total cases	*P. vivax*	*P. falciparum*	SPR	SFR
Jan	41	11	8	3	26.95	7.31
Feb	53	13	9	4	24.52	7.54
Mar	39	11	9	2	28.20	5.12
Apr	65	12	8	4	18.46	6.15
May	47	12	9	3	25.53	6.38
June	67	17	11	6	25.37	8.95
July	89	28	21	7	31.46	7.86
Aug	143	44	31	13	30.76	9.09
Sept	247	136	94	42	55.06	17.00
Oct	293	138	81	57	47.09	19.45
Nov	182	62	41	21	34.06	11.53
Dec	103	14	7	7	13.59	6.79

Table 3 Prevalence of malaria showing parasite distribution, SPR and SFR during year 2003.

Month	BSE	Total cases	*P. vivax*	*P. falciparum*	SPR	SFR
Jan	37	8	6	2	21.62	5.40
Feb	31	11	9	2	35.48	6.45
Mar	51	10	7	3	19.60	5.88
Apr	72	16	11	5	22.2	6.94
May	61	14	10	4	22.95	6.55
June	57	12	9	3	21.05	5.26
July	95	21	14	7	22.10	7.36
Aug	149	53	39	14	35.5	9.39
Sept	218	134	92	42	61.46	19.26
Oct	347	174	102	72	50.14	20.17
Nov	213	106	55	51	49.76	23.94
Dec	97	21	9	12	21.64	12.37

Table 4 Prevalence of malaria *P. vivax* and *P. falciparum* infections during 2001–2003.

Years	BSE	Total positive cases	*P. vivax*	*P. falciparum*
2001	1473	487	311(63.86%)	176(36.13%)
2002	1369	498	329(66.06%)	169(33.93%)
2003	1428	580	363(62.58%)	217(37.41%)

Table 5 shows year wise analysis of *P. vivax,* non-relapsing versus relapsing patients and relapse rates with different follow up duration. In 2001 a total of 144 patients were observed of which 122 did not have any further relapse whereas 22 had relapses in two year follow up study having a relapse rate of 15.2%. Similarly for the year 2002 and 2003 the relapse rates

Table 5 Relapse cases in *P. vivax* infection.

Years	Total no. of patients observed	Non-relapsing patients	Relapsing patients	Short term relapse	Long term relapse	Relapse rate	Follow -up year
2001	144	122	22	16	6	15.2	2
2002	178	144	34	28	6	19.1	2
2003	204	169	35	27	8	17.1	1
2003	88[a]	84	4	4	–	4.5	1

[a] Patients given 75 mg primaquine base following chloroquine treatment.

Table 6 Chloroquine resistant cases in *P. falciparum* infection.

Years	No. of *P. falciparum* patients observed	Non-resistant patients	Resistant patients				Resistance rate			
			Total	RI	RII	RIII	Total	RI	RII	RIII
2001	124	112	12	8	4	–	9.6	6.45	3.2	–
			16	10	6	–	10.8	6.75	4	–
2003	157	139	18	13	5	–	11.4	8.2	3.2	–

were 19.1% and 17.1%, respectively. Short term relapses were more then the long term in the ratio of about 4:1. In 2001–2003 short and long term relapses were recorded as 11.1% and 4.1%, 15.7% and 3.3% and 13.2% and 3.92%, respectively. Above percentage of relapse were recorded in patients who were given 1500 mg chloroquine base in divided doses (i.e. 600 mg on day 1 and 300 mg after 8 h followed by 300 mg daily for 2 days). Eighty-eight patients who were administered with 1500 mg chloroquine and 75 mg primaquine in divided doses also showed resistance in 4.5% cases.

Table 6 shows the frequency distributions of 124,148 and 157 patients showing resistance against chloroquine in 9.6%, 10.8% and 11.4% cases after getting 1500 mg chloroquine base in divided doses (i.e. 600 mg on day 1 and 300 mg after 8 h followed by 300 mg daily for 2 days). Out of 124 patients studied during 2001, 112 were susceptible, whereas 12 patients showed resistance against chloroquine. The level of resistance was RI and RII type in 6.4% and 3.2% cases. In 2002 out of 148 patients 132 were susceptible while 16 showed resistance out of which 6.7% were of RI type and 4.1% were of RII type. Similarly in the year 2003 out of 157 patients observed 139 were found susceptible and 18 showed resistance out of which 8.2% were of RI type and 3.2% were of RII type.

4. Discussion

Blood smears of patients attending hospitals and clinics of Aligarh showing symptoms such as high fever, severe headache, loss of appetite and occasional vomiting were examined microscopically for *P. vivax* and *P. falciparum* infections. Resistant and relapse cases were observed and followed up in *P. falciparum* and *P. vivax* infections, respectively.

In the present study peak transmission of malaria during the months of September and October which was recorded high during the years 2001–2003 was probably because of the availability of plenty of water bodies, supporting breeding of vector species after rainy season in preceding months. Moderate temperature (i.e. 26–28 °C) and optimum humidity (i.e. 77–88%) during these months which were ideal for the mosquito breeding might have influenced increase in vector population which must have contributed to maximum transmission as earlier observed by Adak et al. (1998) in Delhi. Increased transmission of malaria was also recorded during peak season in Assam in areas having streams where vector species which happens to be *Anopheles minimus* was in abundance (Wajihullah et al. 1992). Whereas least transmission which was recorded from December to June in the present study was because of adverse environmental factors which caused reduction in number of vector species. During these months either extreme dry cold or dry heat conditions prevail which affect vectors adversely either by making water bodies unsuitable or very slow for the breeding or by making them scarce as a result of high temperature which even kill the vector.

Percentage for *P. vivax* during years 2001–2003 was 63.44, 66.06 and 62.58 while for *P. falciparum* it was 36.55, 33.93 and 37.41. This indicates decrease in *P. vivax* and an increase in *P. falciparum* infection. Development of resistance against routine curative drug (i.e. chloroquine) might have been one of the reasons for this increase in *P. falciparum*. Slide *falciparum* rate (SFR) was considerably high during peak transmission period as well as in colder months even when transmission rate is low. It is difficult to draw exact conclusion but it seems that development of *P. falciparum* is supported by the vector species more readily as compared to *P. vivax*, may be it is a particular strain of some vector species which emerges in good number in winters and contributed for raised *falciparum* malaria. Another probable explanation may be this that complicated cases are generally referred to medical college and a few of them happens to be *falciparum* malaria, might have contributed to raise number of cases. Since we have collected maximum cases from medical college, SFR obtained in the present study is bit high. In contrast *P. vivax* cases are generally treated outside easily cured and therefore are not on record, hence its transmission rate, which is observed during this study is proportionately low.

Relapse rate in *P. vivax* during the year 2001–2003 following the administration of 1500 mg chloroquine base were 15.2%, 19.1% and 17.1%, respectively. Short-term relapses were more as compared to the long term relapses, which were 11.1%, 15.7%, 13.23% and 4.1%, 3.3%, 3.92%. But relapse rate was quite low (i.e. 4.5%) and was of short term type, when 75 mg primaquine base was also administered following chloroquine treatment. This indicates that both chloroquine and

primaquine were not fully effective even when they were administered in a total curative dose to the patients suffering from vivax malaria. Similar observations were made by Srivastava et al. (1996) who recorded more short-term relapses and a few long-term relapses after administration of 600 mg chloroquine, is in accordance of our finding where more short-term relapses recorded. Adak et al. (1998) recorded 23–44% relapsing cases within a period of 5 years study after giving 900 mg chloroquine in two doses (600 + 300 mg). Smoak et al. (1997) observed relapses in 43% Somalian soldiers who received standard dose of primaquine. In these studies relapse rates were high as compared to our findings. It may be because of the reason that anti relapse drug, primaquine was not administered by Adak et al. while in case of Smoak et al.(1997) study, may be strain of *P. vivax* was more tolerant to anti relapse drug, primaquine and did not respond to it in a fairly good number of cases.

In the present study 9.6%, 10.8% and 11.4% patients suffering from *falciparum* malaria showed resistance by showing recrudescence around first and second week following chloroquine treatment during 2001–2003. Sharma (1999) reported 30% resistant cases in high transmission zone in India. Many other reports are available in India and abroad which show increasing trend of drug resistance. Ghosh et al. (1992) observed 25% chloroquine resistant cases in Orissa. Shah et al. (1997) reported chloroquine resistant cases in Pakistan and Afghanistan. Mharakurwas et al. (1997) reported resistance in as much as 52% patients following chloroquine treatment. Bojang et al. (1998) reported failure of chloroquine in Gambian children. In present study less percentage of resistant cases were recorded which might be because of the reason that study was conducted in low transmission region where *P. falciparum* infection is proportionately less.

Regarding the interpretation of the results, one may disagree with the differentiation of primary attack versus relapse or reinfection, particularly during peak transmission season. However, in the absence of any clinical or parasitological marker, the following observations are considered as relevant. In the present study, malaria cases detected between December and June (the supposed non-transmission season) could be grouped in three categories.

(I) Infections acquired in the previous transmission season i.e. between July and November but remained undetected and thus untreated.
(II) Infections acquired during the previous transmission season that were detected, treated and subsequently reappeared (relapse).
(III) Infections acquired during the previous transmission season that become clinically and parasitologically positive after a prolonged period (delayed primary attack).

Although the reinfection, particularly during the main transmission season could not be ruled out, real transmission from December to June is probably occasional and at a very low level, if supposed non-transmission period with a definite history of malaria were considered to be relapse rather than reinfections, whereas those detected with a history of malaria were considered delayed primary attacks. However, some uncertainty still exists regarding the possibility of reinfection during the supposed non transmission season, which probably will persist until some diagnostic tool is developed that can distinguish the primary attack and subsequent relapse from reinfection.

It is quite evident from the duration of follow up study (1–2 years) that the average resistance rate for the year 2001 was 9.6, 2002 was 10.8 and for the year 2003 was 11.4. The relapse rates for the years 2001–2003 were 15.2, 19.1 and 17.1, respectively. Therefore the present drug policy of National Malaria Eradication Programme for the administration of chloroquine in *P. falciparum* as the first line curative drug and primaquine 15 mg of base, once a day for five consecutive days as anti relapse drug for the radical cure of *P. vivax* infection warrants its reconsideration.

In the view of this information, it is suggested that the frequency distribution/ratios of different parasite forms responsible for different relapse patterns should be determined in different *P. vivax* ecosystems with reference to space and time, which are probably not constant and likely to be time dependent. In addition, the degree to which these parasites variants interact with each other will no doubt have an impact on the maintenance of genetic diversity and regulation of the parasite population as a whole. However, in the absence of parasitological and clinical markers, it may be difficult to characterize these forms; perhaps amplification of specific DNA sequences by the polymerase chain reaction using specific oligonucleotide probes from different parasites isolates of relapsing and non-relapsing patients could be used to analyse the genetic diversity of the *P. vivax* population and correlate this with epidemiological findings. Therefore there is a strong need for integrated laboratory and fields studies as well as the use of mathematical models to interpret the complex transmission dynamics of *P. vivax* and *P. falciparum* so that appropriate malaria control strategies, including chemotherapeutic measures can be devised to prevent relapse and resistance problems in *P. vivax* and *P. falciparum*.

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